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Effects of Tributyltin on Survival, Growth, Morphometry, and RNA-DNA Ratio of Larval Striped Bass, *Morone saxatilis*

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Abstract. Effects of tributyltin (TBT) on survival, growth, morphometry, and RNA-DNA ratio in larval striped bass (*Morone saxatilis*) were assessed in three experiments. In Experiment I, 13 day old larvae were exposed to 0, 0.067, 0.766, or 2.284 μg TBT/L for 6 days. All larvae exposed to 2.284 μg/L died by day 5; exposure to 0.766 μg/L significantly reduced survival relative to controls (59.8% vs. 81.8%). Significant, concentration-dependent decreases in body depth occurred in larvae exposed to 0.067 and 0.766 μg/L. In Experiment II, all 16 day old larvae exposed to 1.498 μg/L died by day 6. Survival, weight, and morphometry parameters were not significantly different in larvae exposed to 0, 0.052, or 0.444 μg/L for 7 days. In Experiment III, survival was similar in 21 day old larvae exposed to 0, 0.055, 0.218, or 0.514 μg/L for 7 days. Notochord length and dry weight decreased significantly in larvae exposed to 0.514 μg/L. Weight and morphometry parameters were more sensitive indicators of sublethal stress than RNA-DNA ratio. Maximum TBT concentrations reported in Chesapeake Bay marinas are likely to cause increased larval mortality. Longer-term studies are needed to assess effects at <0.050 μg/L, which may be more representative of habitat conditions.

Tributyltin (TBT) compounds are introduced into the aquatic environment through their use in antifouling paints on boats and ships and in coatings on fish farming nets. TBT compounds are the subject of international concern because of their toxicity to non-target organisms at low and sub-part per billion concentrations (Hall and Pinkney 1985). The use of TBT has been linked to poor survival and shell abnormalities in the Pacific oyster (*Crassostrea gigas*) in France, Great Britain, and the United States (Alzieu 1986; Thain and Waldock 1986; Wolniakowski *et al.* 1987). TBT has been detected in the edible tissues of chinook salmon (*Oncorhynchus tshawytscha*) and Atlantic salmon (*Salmo salar*) raised in treated net pens and deaths have been reported when fish were transferred to newly treated nets (Short and Thrower 1986; Davies and McKie 1987).

The objective of the present study was to assess the effects of TBT on the survival and growth of larval striped bass (*Morone saxatilis*). The growth rate of larval fish determines the duration of this life stage (Buckley 1984); larval growth is one of the most sensitive indicators of toxicant-induced stress in fish (Norberg and Mount 1985). There are few data on the effects of TBT on fish growth. Seinen *et al.* (1981) reported significant decreases in growth in rainbow trout (*Salmo gairdneri*) exposed to sublethal, nominal concentrations of 0.2 and 1 μg TBT chloride/L for 110 days beginning at the yolk sac fry stage. Mean body weights of these respective groups were 74.5% and 50.9% of the weights of the control group. Hall *et al.* (1988a) exposed 17–19 day old inland silverside (*Menidia beryllina*) larvae to 0, 0.093, or 0.490 μg TBT/L for 28 days. There were significant decreases (20.6–21.6%) in wet weight of TBT-exposed larvae relative to controls. No differences were found, however, in morphometric measurements of notochord length, head length, eye diameter, head depth, or body depth. Ward *et al.* (1981) reported no changes in the length or weight of adult sheepshead minnow (*Cyprinidon variegatus*) exposed to 0.18–1.0 μg TBTO/L for 167 days. Newton *et al.* (1985) found no differences in hatch success or notochord length at hatch in fertilized California grunion (*Leuresthes tenuis*) eggs exposed to 0–2.00 μg TBT/L until hatch (10 days).

Striped bass were chosen for their commercial and recreational importance, sensitivity to organic and inorganic toxicants, and to serve as a model for anadromous species that have suffered population declines in the Chesapeake Bay (Palawski *et al.* 1985; Speir 1987). Early life stages of striped bass may be exposed to TBT in the Chesapeake, where maximum concentrations of 1.801, 0.048, and 0.024 μg/L have been detected in marinas, receiving waters, and open waters, respectively (Hall *et al.* 1987, 1988b). These concentrations exceed the U.S. Environmental Protection Agency's Aquatic Life Advisory Concentration of 0.010 μg TBT/L for salt water (US Environmental Protection Agency 1987). Research reported in this paper examined the effect of larval age on sensitivity to TBT and compared morphom-

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Table 1. Water quality data

Experiment	Temp. (°C)	Salinity (ppt)	D.O. (mg/L)	pH	NH_4-N (mg/L)
I					
Holding[a]	17.0–19.0	0.5–1.5	6.6–7.9	7.74	—
Exposure	18.0–20.0	1.1–3.0	8.3–9.3	7.81–8.47	0.085–0.107
II					
Holding[b]	17.0–19.0	0.5–1.5	7.0–8.1	—	—
Exposure	18.5–19.5	1.9–3.0	6.0–8.9	8.19–8.33	0.047–0.085
III					
Holding[c]	18.5–19.5	1.0–13.0	6.4–8.1	—	0.102
Exposure	20.0–22.0	12.2–14.5	7.5–8.4	7.70–7.75	0.073–0.216

[a] Larvae obtained from hatchery at one day of age; held at CBL for 12 days
[b] Larvae obtained from hatchery at 12 days of age; held at CBL for 4 days
[c] Larvae obtained from hatchery at 10 days of age; held at CBL at 1.0 ± 0.5 ppt salinity for 5 days, then salinity raised to 13.0 ppt over 6 days

etry, condition factor, and RNA-DNA ratio as indicators of larval growth and condition.

Materials and Methods

General Procedures

Three batches of striped bass (*Morone saxatilis*) larvae were obtained from the Cedarville, MD hatchery. Larvae were maintained at the Chesapeake Biological Laboratory in 1000 L tanks under flowing water. Holding water was a mixture of well water and 5 μm filtered Chesapeake Bay water. Data on the age of the larvae and the water quality parameters for the holding and experimental periods are given in Table 1. Larvae over the age of 4 days were fed newly hatched *Artemia* nauplii (2,000 nauplii/L) three times a day.

Tributyltin methacrylate painted panels were obtained from the U.S. Navy, David Taylor Naval Ship Research and Development Center (Annapolis, MD) and installed in a flow-through toxicity testing apparatus (Pinkney *et al.* 1987). Glass aquaria (37.8 L) were lined with 0.254 mm polycarbonate film (applied with silicone sealant) to minimize adsorption. Larvae were exposed in 335 μm nylon-screened 8-L polycarbonate trays, which were suspended in the aquaria such that 4.5 L of water was contained. The system was allowed to run for two weeks prior to use in order to allow TBT concentrations to stabilize.

Three experiments were performed. In Experiment I, 13 day old larvae were exposed to TBT for 6 days; Experiment II, 16 day old larvae were exposed to TBT for 7 days; Experiment III, 21 day old larvae were exposed for 7 days. Experiments I and II were initiated by placing 25 larvae in each of four replicate aquaria at three concentrations of TBT plus a control. For Experiment III, there were two replicates of 25 larvae. Dilution water was a mixture of well water and 5 μm filtered Chesapeake Bay water for Experiments I and II. For Experiment III, 5 μm filtered Bay water was used. Newly-hatched *Artemia* nauplii (2,000 nauplii/L) were added to the tanks three times daily. In Experiment III, the fish also received a larval food supplement (AP-100; Ziegler Brothers, Gardners, PA) once a day. Survival was evaluated daily while the tanks were being cleaned of dead fish and debris.

Analytical Procedures

Water samples were collected from tanks at each concentration on days 0 and 4 for Experiment I, days 0, 4, and 7 for Experiment II, and days 1 and 7 for Experiment III. Di- and tributyltin concentrations (Table 2) were determined according to the atomic adsorption hydride derivatization method of Valkirs *et al.* (1986). A Perkin-Elmer 403 spectrometer equipped with an electrodeless discharge lamp (λ = 286.3 nm) was used. Peak areas were quantified with a Hewlett-Packard 3410 Integrator.

Table 2. Measured TBT^+ and DBT^{++} concentrations (mean ± standard deviation) for Experiments I–III

Experiment	TBT^+ (μg/L)	DBT^{++} (μg/L)
I[a]	<0.030 (n = 7)	0.015 ± 0.005 (n = 7)
	0.067 ± 0.026 (n = 7)	0.023 ± 0.008 (n = 7)
	0.766 ± 0.220 (n = 6)	0.158 ± 0.085 (n = 6)
	2.284 (n = 1)	0.295 (n = 1)
II[b]	<0.030 (n = 8)	0.018 ± 0.010 (n = 8)
	0.052 ± 0.019 (n = 8)	0.025 ± 0.025 (n = 8)
	0.444 ± 0.077 (n = 9)	0.080 ± 0.021 (n = 7)
	1.498 ± 0.252 (n = 7)	0.219 ± 0.086 (n = 5)
III[c]	<0.030 (n = 4)	0.023 ± 0.017 (n = 4)
	0.055 ± 0.023 (n = 3)	0.033 ± 0.007 (n = 3)
	0.218 ± 0.107 (n = 3)	0.037 ± 0.005 (n = 3)
	0.514 ± 0.097 (n = 3)	0.103 (n = 2)[d]

[a] Samples collected on days 0 and 4 except for the highest concentration (day 0 only)
[b] Samples collected on days 0, 4, and 7
[c] Samples collected on days 1 and 7
[d] Mean of 0.042 and 0.164; collected on day 7

At the end of Experiments I and II, half of the fish from each tank were stored at −70°C for RNA and DNA determinations. There were insufficient larvae for RNA and DNA determinations in Experiment III. Four fish from each tank were placed in scintillation vials in 5% formalin buffered with $CaCO_3$ for one week and then stored in 2.5% $CaCO_3$ buffered formalin for morphometry and dry weight (DW) determinations.

RNA and DNA determinations were performed on aqueous homogenates of two larvae from Experiment I and of single larvae for Experiment II, according to methods described in Wright and Martin (1985). The absorbance at 260 nm of the acid-soluble, alkali-hydrolyzed fraction was used to determine RNA content. Absorbance (260 nm) of the alkali-stable, acid-hydrolyzed fraction was used for DNA determination.

The morphometry procedures of Martin and Malloy (1980) were followed. Preserved fish were measured for: (1) notochord length (NL), (2) head length (HL), (3) eye diameter (ED), (4) head depth

(HD), (5) body depth at the base of the pectoral fin (BDP), and (6) body depth at anus (BDA) (Figure 1). Measurements were made with a dissecting microscope and ocular micrometer at 7× on 16 fish/group for Experiments I and II and 8 per group for Experiment III. Afterwards, the fish were dried in a 60°C oven for five days and weighed to the nearest μg on a Cahn electrobalance. The condition factor (CF) was determined by the formula $CF = (DW/NL^4) \times 10^5$ (based on data of Houde and Lubbers 1986).

Statistics

The arc sine square root transformation was used in the analysis of the survival data. Data were analyzed as randomized block designs with replicate tanks as blocks. Significance testing was performed by one way ANOVA followed by Duncan's Multiple Range Test (Sokal and Rohlf 1981; SAS Institute 1985). For the morphometry, weight, condition factor and RNA-DNA data, ANOVA was performed with tanks nested within each concentration (Sokal and Rohlf 1981). Duncan's Multiple Range Test was performed to analyze for differences between groups (SAS Institute 1985). For all procedures, the p value of 0.05 was used for significance testing.

Results

In Experiment I, all larvae exposed to an average concentration of 2.284 μg TBT/L died within 5 days (Figure 2). Survival was significantly reduced in larvae exposed to 0.766 μg TBT/L (59.8%) relative to controls (81.8%). In Experiment II, there was 100% mortality by day 6 in larvae exposed to an average concentration of 1.498 μg TBT/L (Figure 2). Few deaths occurred in the first 48 hr of either experiment. There were no significant differences in the survival of larvae exposed to 0–0.514 μg TBT/L in Experiment III (Figure 2).

The sequence of behavioral toxic signs was similar in the fish that died in the highest concentration tanks in Experiments I and II. The first sign was slowed swimming. Later, larvae lost equilibrium and remained motionless on the bottom. If prodded, such larvae would swim erratically, sometimes in spirals, and then fall to the bottom again. Fish often remained motionless on the bottom for up to 24 hours before dying. No signs of toxicity were observed in the larvae exposed to 0.766 μg TBT/L in Experiment I.

In larvae exposed to 0.067 μg TBT/L in Experiment I, there were significant decreases in the two body depth measurements. BDP decreased by 4.8% while BDA decreased by 8.6%. Significant decreases in BDP (−11.0%), BDA (−16.3%), HL (−10.0%), and DW (−20.7%) occurred in the larvae exposed to 0.766 μg TBT/L (Table 3).

No significant differences were found in morphometry, dry weight, condition factor, and RNA-DNA data in control vs. exposed larvae from Experiment II (Table 4). In Experiment III, there were significant reductions in NL (−6.2%) and DW (−19.2%) at the 0.514 μg TBT/L concentration (Table 5).

Discussion

Concentrations ≥1.498 μg TBT/L caused 100% lethality in larval striped bass in 5–6 days. Striped bass appear to be in the same range of sensitivity to TBT as other larval fish. Ninety-six-hr LC50s of 2 μg TBTO/L (nominal concentration) for sole (*Solea solea*) (Thain 1983), and 3.0 μg TBT/L for inland silverside (*Menidia beryllina*) (Bushong *et al.* 1987), have been reported.

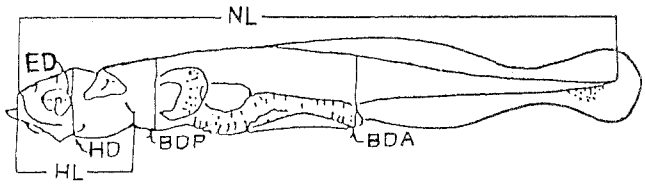


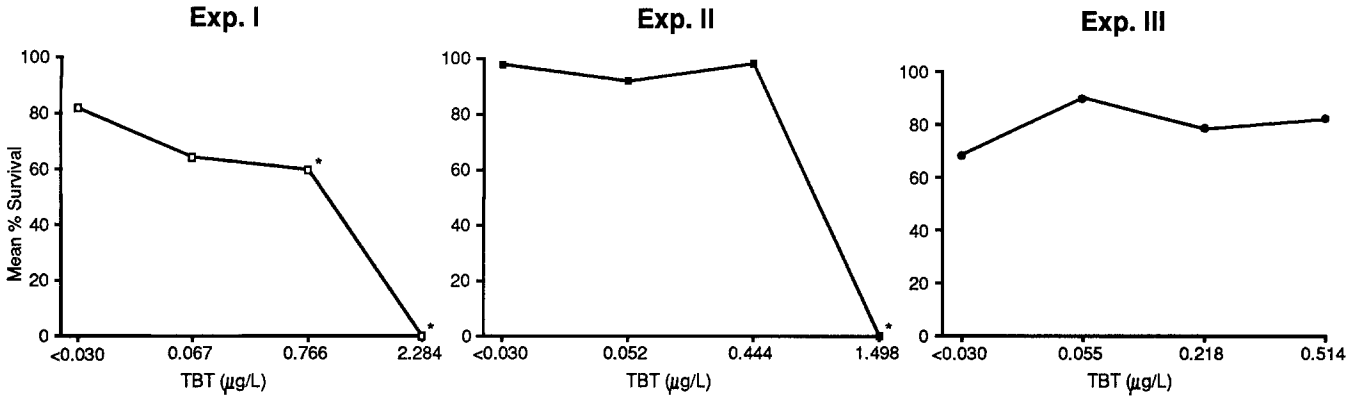
Fig. 1. Morphometric measurements performed on larval striped bass (NL = Notochord length; ED = Eye diameter; HD = Head depth; HL = Head length; BDA = Body depth at anus; BDP = Body depth at pectoral fin)

At concentrations ≥0.766 μg TBT/L, larval survival was significantly reduced. Hall *et al.* (1988b) reported maximum water column concentrations of 1.801 and 1.171 μg TBT/L in Chesapeake Bay marinas in June samples. The laboratory mortality data of this study indicate that exposure of striped bass larvae to those marina concentrations would be likely to result in increased mortality.

Several morphometric parameters and larval dry weight were reduced in 13 day old larvae exposed to 0.766 μg TBT/L in Experiment I. These data should be viewed with caution, however, since there was a significant decrease in survival at this concentration. The lack of growth in these fish may therefore be a prelude to the imminent death of the larvae. Decreases in notochord length (−6.2%) and dry weight (−19.2%) were also observed in 21 day old larvae exposed to 0.514 μg TBT/L. Similarly, Hall *et al.* (1988a) reported a decrease in wet weight (−20.6%) of larval *Menidia beryllina* exposed to 0.490 μg TBT/L for 28 days. These data suggest that TBT concentrations in the 0.5–0.8 μg/L range, which have been reported in marinas of the Chesapeake (Hall *et al.* 1987, 1988b) may be detrimental to the survival and growth of larval fish.

The decreases in the body depth parameters (BDA and BDP) in 13-day old larvae exposed to 0.067 μg TBT/L in Experiment I may be indicative of sublethal growth effects, although there were no significant changes in dry weight. These results are comparable with those of Hall *et al.* (1988a) who reported decreased wet weight in larval *M. beryllina* exposed to 0.093 μg TBT/L for 28 days but no changes in any morphometry parameters. However, the decreases observed in 13 day old larvae in Experiment I were not observed in either 16 day or 21 day larvae exposed to similar concentrations in Experiments II or III. These contrasting results may reflect differences in the sensitivities of the different batches of larvae or an increased sensitivity of the younger larvae.

Dry weight, body depth, head length, and notochord length were the most sensitive parameters to the effects of TBT. Condition factor was notably insensitive. Condition factor actually increased in Experiment III fish exposed to 0.514 μg TBT/L, while notochord length and dry weight decreased. RNA/DNA ratio was decreased but not signifi-



* Survival significantly reduced relative to control survival ($p<0.05$, ANOVA, Duncan's)

Fig. 2. Striped bass larval survival in Experiments I–III

Table 3. Mean ± standard error of morphometry, dry weight, condition factor and RNA/DNA data for Experiment I[a]

	TBT⁺ (µg/L)		
Parameter[b]	<0.030	0.067	0.766
NL (mm)	13.66 ± 0.16	13.39 ± 0.22	13.05 ± 0.28
HL (mm)	3.42 ± 0.06 (A)[c]	3.26 ± 0.09 (A, B)	3.08 ± 0.09 (B)
ED (mm)	1.08 ± 0.01	1.07 ± 0.06	0.99 ± 0.02
HD (mm)	2.62 ± 0.03	2.61 ± 0.08	2.43 ± 0.05
BDP (mm)	2.74 ± 0.04 (A)	2.61 ± 0.07 (B)	2.44 ± 0.06 (C)
BDA (mm)	2.45 ± 0.05 (A)	2.24 ± 0.08 (B)	2.05 ± 0.08 (C)
DW (mg)	0.82 ± 0.04 (A)	0.76 ± 0.05 (A)	0.65 ± 0.04 (B)
CF	2.33 ± 0.05	2.32 ± 0.05	2.23 ± 0.10
RNA/DNA	2.30 ± 0.12[d]	2.29 ± 0.19[e]	2.15 ± 0.07[e]

[a] Nested ANOVA based on 4 larvae/tank, 4 tanks/concentration
[b] Notochord length (NL), head length (HL), eye diameter (ED), head depth (HD), body depth at pectoral fin (BDP), body depth at anus (BDA), dry weight (DW), condition factor (CF)
[c] Duncan grouping; groups with different letters are significantly different ($p < 0.05$)
[d] Based on 2–4 larvae/tank; 4 tanks/concentration
[e] Based on 1–4 larvae/tank; 4 tanks/concentration

Table 4. Mean ± standard error of morphometry, dry weight, condition factor and RNA/DNA data for Experiment II[a]

	TBT⁺ (µg/L)		
Parameter	<0.030	0.052	0.444
NL (mm)	14.72 ± 0.21	14.96 ± 0.16	14.98 ± 0.20
HL (mm)	3.76 ± 0.08	3.74 ± 0.06	3.65 ± 0.09
ED (mm)	1.13 ± 0.02	1.16 ± 0.02	1.15 ± 0.02
HD (mm)	2.87 ± 0.05	2.86 ± 0.05	2.93 ± 0.05
BDP (mm)	2.97 ± 0.05	2.87 ± 0.06	2.96 ± 0.06
BDA (mm)	2.88 ± 0.08	2.85 ± 0.10	2.81 ± 0.08
DW (mg)	1.12 ± 0.06	1.12 ± 0.05	1.09 ± 0.08
CD	2.34 ± 0.40	2.22 ± 0.05	2.13 ± 0.13
RNA/DNA	2.21 ± 0.10[b]	2.24 ± 0.08	2.40 ± 0.11[c]

[a] Nested ANOVA based on 4 larvae/tank; 4 tanks/concentration
[b] Based on 4–7 larvae/tank; 3 tanks/concentration
[c] Based on 3–5 larvae/tank; 4 tanks/concentration

cantly in Experiment I larvae that showed significant decreases in dry weight, head length, and body depth.

Tributyltin can be a slow-acting toxicant, either as a neurotoxic agent or by interfering with energy metabolism (Laughlin and Linden 1985; Aldridge 1976). There are few data on the sublethal effects of TBT on larval fish. In addition to decreased growth, Seinen *et al.* (1981) also reported liver hyperplasia, decreased liver glycogen content, decreased hemoglobin content, and a diminished erythrocyte count after chronic exposure of yolk sac fry rainbow trout at nominal concentrations of 0.2 and 1.0 µg TBT chloride/L. Long-term exposures of larval fish should be performed at measured concentrations of <0.050 µg TBT/L, which may be representative of habitat concentrations. Survival, growth, and morphometry are suitable endpoints. Mechanistic studies should focus on possible effects on the nervous system, energy metabolism, liver histology, and hematology.

Table 5. Mean ± standard error of morphometry, dry weight, and condition factor data for Experiment III[a]

	TBT$^+$ (μg/L)			
Parameter	<0.030	0.055	0.218	0.514
NL (mm)	19.55 ± 0.33 (A)[b]	19.62 ± 0.16 (A)	19.42 ± 0.35 (A)	18.34 ± 0.38 (B)
HL (mm)	5.54 ± 0.14	5.35 ± 0.10	5.48 ± 0.18	5.21 ± 0.12
ED (mm)	1.60 ± 0.02	1.57 ± 0.02	1.57 ± 0.03	1.56 ± 0.02
HD (mm)	4.18 ± 0.03	4.16 ± 0.07	4.29 ± 0.07	4.16 ± 0.07
BDP (mm)	4.45 ± 0.12	4.48 ± 0.09	4.31 ± 0.07	4.27 ± 0.12
BDA (mm)	4.36 ± 0.08	4.48 ± 0.08	4.31 ± 0.10	4.20 ± 0.10
DW (mg)	3.70 ± 0.23 (A)	3.62 ± 0.15 (A)	3.41 ± 0.24 (A, B)	2.99 ± 0.28 (B)
CD	2.52 ± 0.08	2.43 ± 0.05	2.38 ± 0.07	2.58 ± 0.08

[a] Nested ANOVA based on 4 larvae/tank; 2 tanks/concentration
[b] Duncan grouping; groups with different letters are significantly different ($p < 0.05$)

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